

Variability in macrozoobenthic assemblages along a gradient of environmental conditions in the stream water of karst caves (Lower Shakuranskaya Cave, western Caucasus)

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Academic editor: Rodrigo Lopes Ferreira | Received 10 March 2021 | Accepted 1 July 2021 | Published 23 July 2021

<http://zoobank.org/F1A78CFB-D7E5-4417-B7D8-2634185705D3>

Citation: Borisov RR, Chertoprud ES, Palatov DM, Novichkova AA (2021) Variability in macrozoobenthic assemblages along a gradient of environmental conditions in the stream water of karst caves (Lower Shakuranskaya Cave, western Caucasus). *Subterranean Biology* 39: 107–127. <https://doi.org/10.3897/subtbiol.39.65733>

Abstract

The fauna of the stream water in the Lower Shakuranskaya Cave in central Abkhazia, western Caucasus, was studied. This cave has a large inlet and an extended entrance ecotone area of approximately 60 m, which makes it a convenient area for studying macrozoobenthic assemblages across a gradient of environmental factors. The cave has 13 species of stygobionts, 10 species of stygophiles and 18 species of stygoxenes. The number of species and the abundance and biomass of stygobionts per station were the highest near the boundary of the photic zone, at a distance of 50–60 m from the entrance to the cave, and gradually decreased toward both the remote parts of the cavity and the cave exit. The most abundant stygobionts were gastropod mollusks of the Hydrobiidae family, and *Xiphocaridinella* shrimp comprised the main part of the biomass. It has been shown that the main environmental factors determining the distribution of macrozoobenthos are luminosity and distance from the entrance to a cave. According to the differences in their reactions to these environmental factors, several groups of species were identified. In addition, three main assemblages of macrozoobenthic species were described: (1) an assemblage of epigean species near the cave entrance area; (2) stygobionts in remote parts of the cave outside the photic zone; and (3) a mixed assemblage in the cave ecotone, where a faint light penetrates. The specific details related to the faunal structure in the ecotone of the cave are discussed, as well as active and passive methods by which stygoxenes invade underground cavities.

Keywords

Abkhazia, distribution, ecological factors, species richness, stygobionts, stygophiles, stygoxenes

Introduction

Natural communities are usually not discrete but gradually change each other under the influence of environmental factors (Riesch et al. 2018). The values of environmental factors at both local and geographical scales often have a pronounced gradient. Appreciating that ecological gradients prevail in nature allows us to look at the distribution of organisms from the standpoint of continuity. A question that has a broad interest is how fauna and community structures change along ecological gradients.

Caves can serve as a model system for studying community variation along an environmental gradient at a local spatial scale. The entrances of caves are transition zones where epigeic and endogeic organisms can encounter each other. These ecotones are rich in food due to primary producers and accumulated debris from epigeic ecosystems, especially in comparison to the food in deeper parts of the same systems (Pentecost and Zhaohui 2001; Culver and Pipan 2009). However, the variability in the community structures in streams on a gradient of epigeic-endogeic conditions has received little attention. Presumably, communities with intermediate epigeic and underground characteristics can live in a cave ecotone. It has been noted that near cave entrances, an invertebrate community may be characterized by higher species richness than that in neighboring epigeal communities or communities deeper in a cave (Prous et al. 2004, 2015). However, the general patterns of changes in aquatic communities along the gradient of environmental factors in caves remain undescribed.

The aquatic invertebrate fauna of caves from the western Caucasus is rich (nearly 110 species) and highly taxonomically specific, with endemics accounting for more than 90% of the species (Kniss 2001; Shumeyev 2008; Sidorov 2014; Vinarski et al. 2014; Barjadze et al. 2015; Sidorov et al. 2015a, b; Turbanov et al. 2016). The large number of caves and, at first glance, the accessibility to explorers could make this region convenient for studying community changes on the gradient from epigeic to underground conditions. However, integrated studies comparing the invertebrate assemblage structures in the different parts of an entire cave are rare for the western Caucasus (Chertoprud et al. 2016, 2020). Studies of the fauna in underground watercourses in the region face a number of problems: the presence of substrates with monolithic slabs, which complicates the collection of data; the inaccessibility of a large part of underground watercourses; and different technical difficulties related to underground research work. Incidentally, the success of such studies is largely due to the choice of a suitable cave system.

The work in this study was devoted to analyzing the structure and spatial distribution of macrozoobenthos assemblages in the watercourse of the Lower Shakurskaya Cave (Abkhazia, western Caucasus). Here, we tested the hypothesis that the macroinvertebrate assemblages in the cave ecotone may significantly differ from the assemblages

es in the remote parts of the cave, both in terms of species composition and structural dominance. We attempted to identify the main factors determining the penetration of epigeic species into the underground cavities.

Materials and methods

Explored area

The research was carried out in the Lower Shakuranskaya Cave, located in the Gulripshi district of Abkhazia, on the orographically right shore of the Jampal River, 1.5 km south of the village of Amtkel. The configuration of this cave allowed us to conduct research on a 650 m long transect, with a focus on the ecotone zone of the cave. The substrate of the Lower Shakuranskaya Cave consists of Late Cretaceous limestones and belongs to the speleological area of the southern slope (speleological province of the Greater Caucasus) of the Gumishkhinsko-Panavsky speleological district (Dublyansky et al. 1987). The total length of the galleries of the accessible part of the cave is approximately 1300 m (Maksimovich 1965; Dublyansky et al. 1987). The water inflow in the cave has a condensation-infiltration origin (Amelichev et al. 2007). The water flow is represented by a stream originating at the deepest part of the cave from a small waterfall (Fig. 1). The cave is characterized by a large number of rimstone dams and pools. Due to the presence of rimstone dams, shallow water areas with a fast current alternate with deep (1–1.5 m) areas with a slow drift. The stream occupies the entire width of the main cave gallery over a considerable length of the cave. The height of the Lower Shakuranskaya Cave entrance is 13 m, and the width is 10 m that, at a distance of 60 meters, decrease to 7 m and 3.5 m, respectively. Illumination penetrates the cave at a distance of 36 m from the entrance (ecotone zone).

Sampling strategy

Sampling stations were set in a transect along the stream course in the main cave gallery. The transect had a length of approximately 650 m and included eight stations located from the deepest halls to the entrance area (Fig. 1). The transect stations were located in areas of the stream with an apparent flow. The studies were carried out at three time points: February 2018 and May and October 2019. In October, three more stations were added from the ecotone zone to the main transect, with eight stations (Fig. 1). In total, 27 quantitative and complex samples of macrozoobenthos were obtained.

The high heterogeneity of the biotopes and low values of faunal abundance and species richness often make it difficult to carry out ecological studies in caves to a full extent. To compose a complete picture of the structure of species assemblages, quantitative complex samples of hydrobionts were obtained at each station (one complex sample per station). Each complex sample included organisms from three sites 3 m away from each other at a given station. At each station, the samples covered both the areas

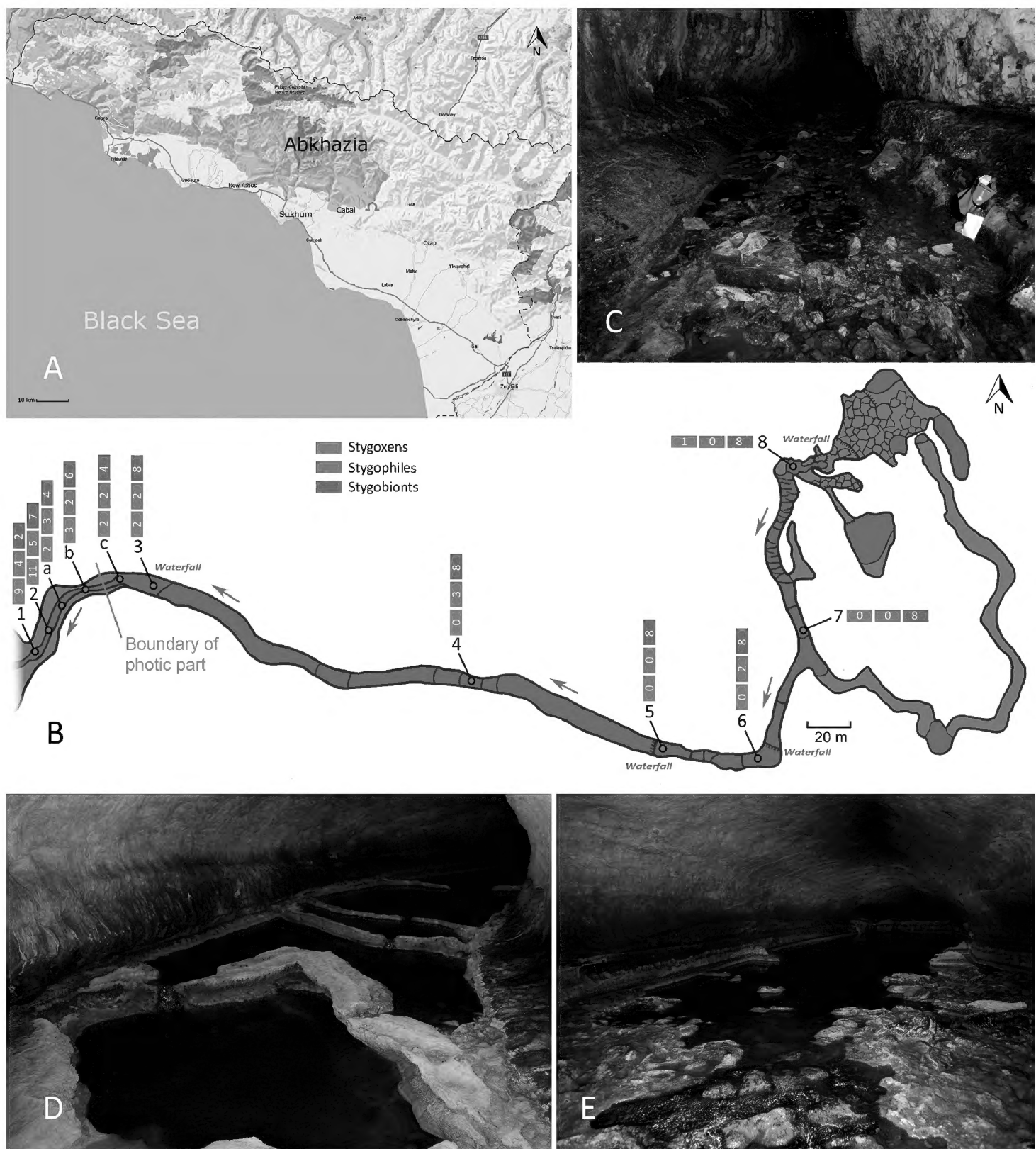


Figure 1. **A** Location of the Lower Shakuranskaya Cave on the map of Abkhazia **B** sampling stations location scheme in the Lower Shakuranskaya Cave in 2018–2019. In numbers – stations, sampled in February 2018, May and October, 2019; in letters – additional stations, sampled in October, 2019 (for each station indicated numbers of stygobionts, stygophiles and stygoxenes). Views on the cave stream **C** at station 2 (ecotone zone) **D** at station 4 **E** at station 5.

with the maximum depths and those at the water edge. The main substrate types at the studied stations were stones and clay sand as well as calcified rimstone walls. Collecting aquatic invertebrates was conducted with a hemispherical sampler (diameter 11 cm) with a mesh size of 0.5 mm. The total area of one complex sample at each station was 0.5 m². All the collected organisms were fixed with 90% ethanol. The species composition, abundance and fresh biomass were determined. The biomass was measured with Acculab ALC-210d4 electronic scales (Germany) with an accuracy of 0.001 mg.

Table 1. The main characteristics of the studied stations in Lower Shakuranskaya Cave. (Temperature and hydrochemical characteristics of water are given for October 2019).

Characteristic	Stations										
	1	2	a	b	c	3	4	5	6	7	8
Substrates*	1	1	1	1	1	2	2	2	2	2	2
Distance from the cave entrance, m	0	12	24	36	48	60	280	380	460	520	650
Illuminance, lx	555	13.17	2.70	0.07	0	0	0	0	0	0	0
Maximum stream depth, m	0.25	0.20	0.20	0.03	0.30	0.40	0.50	0.40	0.30	0.50	0.3
Maximum stream width, m	1.0	1.0	1.0	1.0	2.5	3.0	3.0	3.0	3.0	5.0	4.0
Maximum flow rate, m/s	0.47	0.35	0.30	0.20	0.20	0.30	0.30	0.30	0.30	0.20	0.20
Temperature, °C (October 2019)	12.8	12.8	12.8	12.8	12.8	12.5	12.5	12.5	12.7	12.6	12.9
Mineralization, ppm (October 2019)	176	178	175	170	170	188	211	226	250	250	250
pH (October 2019)	7.7	7.8	7.8	7.8	7.8	7.9	7.9	7.8	7.85	7.9	7.7

*Types of substrates: 1 – stones and clay sand; 2 – stones, clay sand and rimstone walls.

At each station, the main hydrological characteristics of the water inflow (width, depth, water discharge, and type of sediments) and illumination (at midday) were measured (Table 1). In 2019, the water temperature, total mineralization (ppm) and pH were additionally determined (Table 1). The measurements were performed using a Hanna portable water analyzer (HI 98129) and Peak Meter MS6612 luxmeter.

Measurements were obtained by the same person at all stations of a transect. The sampling protocol followed the classic scheme used to study freshwater invertebrates (for example, Walseng et al. 2018).

Ecological groups

In this research, the term “stygon” is used, which is suggested for aquatic underground communities, and the terms “stygebionts”, “stygothiles”, and “stygoxenes” are used for classifying such organisms (Husmann 1966, 1967). The species were classified into three ecological groups on the basis of published data (Kniss 2001; Shumeyev 2008; Sidorov 2014; Vinarski et al. 2014; Barjadze et al. 2015; Sidorov et al. 2015a, b; Turbanov et al. 2016; Chertoprud et al. 2020). According to the scheme by which these ecological groups are differentiated, stygebionts can be distinguished from stygothiles by morphological adaptations to cave habitats. Specific morphological adaptations of stygebionts limit their penetration into epigeal communities, rendering them vulnerable to predators that can see and negative effects of ultraviolet radiation (correct for some groups) (Fišer et al. 2014). Stygothiles, in turn, differ from stygoxenes by possessing ecological adaptations to life in underground cavities, such as the ability to survive and complete their full life cycle in oligotrophic cave environments. Stygoxenes are epigeic organisms trapped in caves for random reasons.

Statistical analysis

To evaluate the effects of environmental factors on the community structure, we used distance-based linear modeling (DistLM) and redundancy analysis (RDA). The analysis was performed twice, for the whole massive of data and separately for the data of 2019

year. Our environmental data contained four variables for the whole dataset (year, season, distance, and luminosity), and six additional variables were included for the set of samples collected in 2019 (maximum depth of the stream, maximum width, flow rate, water temperature, total mineralization (total dissolved solids (TDS) and pH). All the available factors were included to each DistLM test. First, marginal tests were performed to determine the effect of each variable on the variation in species assemblage structure. Then, the best-fitting model was selected using the Akaike information criterion (AICc). This criterion is used to select significant factors in a model and take into account sample size by increasing the relative penalty for model complexity with small data sets. Sequential tests are provided for each variable that is added to the model.

A dbRDA (distance-based redundancy analysis) analysis was used to ordinate the fitted values from a given model. Additionally, the original data were analyzed using the MDS (nonmetric multidimensional scaling) factored with luminosity. The analysis was performed in Primer and Permanova+ PRIMER-E, Plymouth, UK (Clarke and Gorley 2001). The ordination of the samples was performed on the basis of the rank matrix of Bray-Curtis similarities.

Regression analysis was performed to indicate the variation in the number of species along the gradient effect of the environmental factors. We used linear regression analysis in Microsoft Excel (Microsoft, Redmond, WA, USA) for the dataset including number of species at each station and four explanatory factors – season, distance, luminosity and year. The Shannon diversity index was calculated for the samples using Excel too. We also applied the constrained ordination technique canonical correspondence analysis (CCA) to determine the impact of the environmental variables on the invertebrate community and show the variations in the species assemblages in accordance with the observed environmental factors in PAST (Hammer et al. 2001).

Results

Species richness

In total, 42 species of aquatic invertebrates were found in the stream of the Lower Shakuranskaya Cave in 2018–2019: Turbellaria – 2; Oligochaeta – 4; Hirudinea – 1; Gastropoda – 6; Bivalvia – 1; Amphipoda – 5; Decapoda – 2; Ephemeroptera – 3; Plecoptera – 2; Coleoptera – 5; Trichoptera – 7; and Diptera – 4. Among them, 14 species were categorized as stygobionts, 10 as stygophiles, and 18 as stygoxenes based on the available literature data (Table 2). Of the 28 species of stygophiles and stygoxenes, most (21 species) were insects. In the illuminated ecotone zone, 33 species were found; outside the photic zone, 26 species were found. Moreover, only 17 species of aquatic invertebrates were recorded at stations more than 60 m from the cave entrance. The highest species richness (23) was observed at station 2 (Table 2), located 12 m away from the cave entrance. The species richness at stations more than 60 m away from the cave entrance varied from 8 to 11 species per sample. The Shannon diversity index varied from 1.58 to 2.98 and generally decreased from the cave entrance to the deepest parts of the cave (Fig. 2).

Table 2. Distribution of aquatic invertebrates on the transect stations in the stream of the Lower Shakuranskaya Cave in 2018–2019.

Species	Stations										
	1	2	a†	b†	c†	3	4	5	6	7	8
Turbellaria											
² <i>Dugesia taurocaucasica</i> (Livanov, 1951)	***	**	***	**	*	*					
³ <i>Dendrocoelum</i> sp.						*					
Oligochaeta											
² <i>Haplotaxis gordioides</i> (Hartmann, 1821)							*				
² <i>Rhynchelmis</i> sp.							*				
³ <i>Stylodrilus</i> sp.						***	**	***	***	**	*
² <i>Eisenia</i> sp.									*		
Hirudinea											
¹ <i>Haemopsis sanguisuga</i> (Linnaeus, 1758)		*			*						
Gastropoda											
² <i>Tschernomorica caucasica</i> (Starobogatov, 1962)	**	***	***	***	*						
³ <i>Caucasogeyeria horatieformis</i> (Starobogatov, 1962)						**	**	**	**		
³ <i>Pontohoratia birsteini</i> (Starobogatov, 1962)		***	**	***	***	***	***	***	**	*	*
³ <i>Caucasopsis schakuranica</i> (Starobogatov, 1962)						*	**	*	**	**	*
³ <i>Caucasopsis shadini</i> (Starobogatov, 1962)											**
³ <i>Caucasopsis</i> sp.		*								*	*
Bivalvia											
³ <i>Euglesa</i> cf. <i>ljovuschkini</i> (Starobogatov, 1962)		**	**	**	**						
Amphipoda											
³ <i>Niphargus magnus</i> Birstein, 1940	*			*							
³ <i>Niphargus inermis</i> Birstein, 1940		**	*	**	**	**	*	**	*	**	*
³ <i>Niphargus</i> cf. <i>ablaskiri</i> Birstein, 1940								*			
³ <i>Zenkevitchia yakovi</i> Sidorov, 2015		*				**	**	**	**	**	**
² <i>Gammarus</i> cf. <i>komareki</i> (Schaferna, 1922)	****		*								
Decapoda											
³ <i>Xiphocaridinella falcirostris</i> Marin, 2020		*		*			**	***	*	**	
³ <i>Xiphocaridinella osterloffii</i> (Juzbašjan, 1941)	*	***	***	***	***	****	***	**	*	**	*
Insecta											
Ephemeroptera											
¹ <i>Electrogena zimmermanni</i> (Sowa, 1984)			*								*
¹ <i>Baetis</i> (<i>Rhodobaetis</i>) cf. <i>gemellus</i> Eaton, 1885	****	**		*							
Leptophlebiidae											
¹ <i>Paraleptophlebia weneri</i> Ulmer, 1920	*	*									
Plecoptera											
¹ <i>Nemoura martynovia</i> Claasen, 1936		*									
² <i>Leuctra</i> sp.		*							*		
Coleoptera											
¹ <i>Agabus</i> (<i>Gaurodytes</i>) <i>guttatus</i> (Paykull, 1798)	*										
² <i>Limnius colchicus</i> Delève, 1963		*									
¹ <i>Riolus somcheticus</i> (Kolenati, 1846)		*									
¹ <i>Elmis</i> sp.	*										
² <i>Odeles</i> sp.	*						*				
Trichoptera											
¹ <i>Tinodes valvatus</i> Martynov, 1913	*										
² <i>Plectrocnemia latissima</i> Martynov, 1913		*				*					
¹ <i>Chaetopterygella abchazica</i> Martynov, 1916	*	*		*							
¹ <i>Stenophylax clavatus</i> (Martynov, 1916)	*										
¹ <i>Lithax incanus</i> (Hagen, 1859)	**		*								
¹ <i>Ernodes palpatius</i> (Martynov, 1909)						*					
¹ <i>Schizopelex cachetica</i> Martynov, 1913		*		*	**						
Diptera											
¹ <i>Macropelopia</i> sp.		*									
¹ <i>Parametriocnemus</i> sp.		*				*					
¹ <i>Cnetha</i> sp.	*	*									
¹ <i>Dixa submaculata</i> Edwards, 1920		*									
Total number of species	15	23	9	11	8	12	11	9	10	8	9

¹ Stygoxenes, ² Stygophiles, ³ Stygobionts. Occurrence: * – single (1–2 specimens per sample); ** – rarely (3–8 specimens per sample); *** – often (9–26 specimens per sample); **** – frequent (27–80 specimens per sample); † – addition sampling stations taken in October 2019.

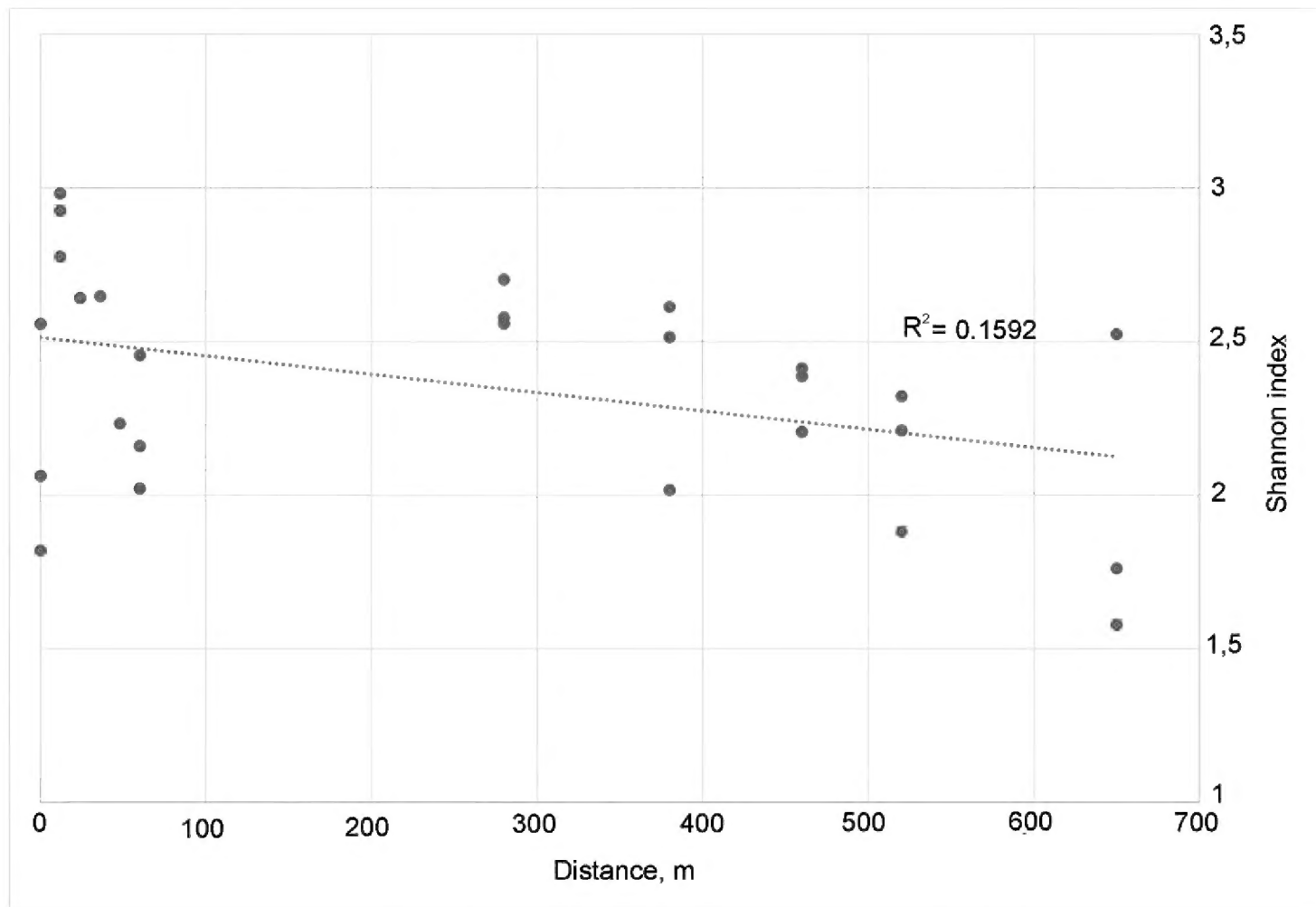


Figure 2. The variation in the values of Shannon diversity index along the distance from the cave entrance (2018–2019).

Number and biomass

The highest abundance values (up to 250 ind/m²) were recorded at the stations in the ecotone zone (Fig. 3). With increasing distance from the cave entrance, a tendency towards decreasing aquatic invertebrate abundance was observed (up to 31 ind/m² at the furthest station of the transect). The most numerous stygobionts in all years of this research were shrimp (*Xiphocaridinella*) and gastropods (*Pontohoratia birsteini*) (Starobogatov, 1962) (both up to 68 ind/m²) (Fig. 4). These species have been recorded throughout the main cave gallery. Another species recorded at all the stations, except for the first station, was *Niphargus inermis* Birstein, 1940. However, its abundance did not exceed 14 ind/m². In addition, oligochaetes (*Stylodrilus* sp.), amphipods (*Zenkevitchia yakovi* Sidorov, 2015), and snails (*Caucasopsis schakuranica* (Starobogatov, 1962) and *Caucasogeyeria horatieformis* (Starobogatov, 1962)) were common outside the photic zone. At station 1, which was outside the cave and had the highest illuminance (555 lx), specimens of stygobiont fauna were found only occasionally. However, at the station located 12 m from the cave entrance (illumination 13 lx), the average proportion of stygobionts was 55% of the total number of invertebrates (Fig. 3). At the ecotone stations, which had slight or no illuminance (0.07 lx), the proportion of stygobionts in the samples increased. At a distance of 36 m from the entrance, stygobionts accounted for 73% of the total number of organisms; at 48 m, 90%; and at 60 meters, 97%.

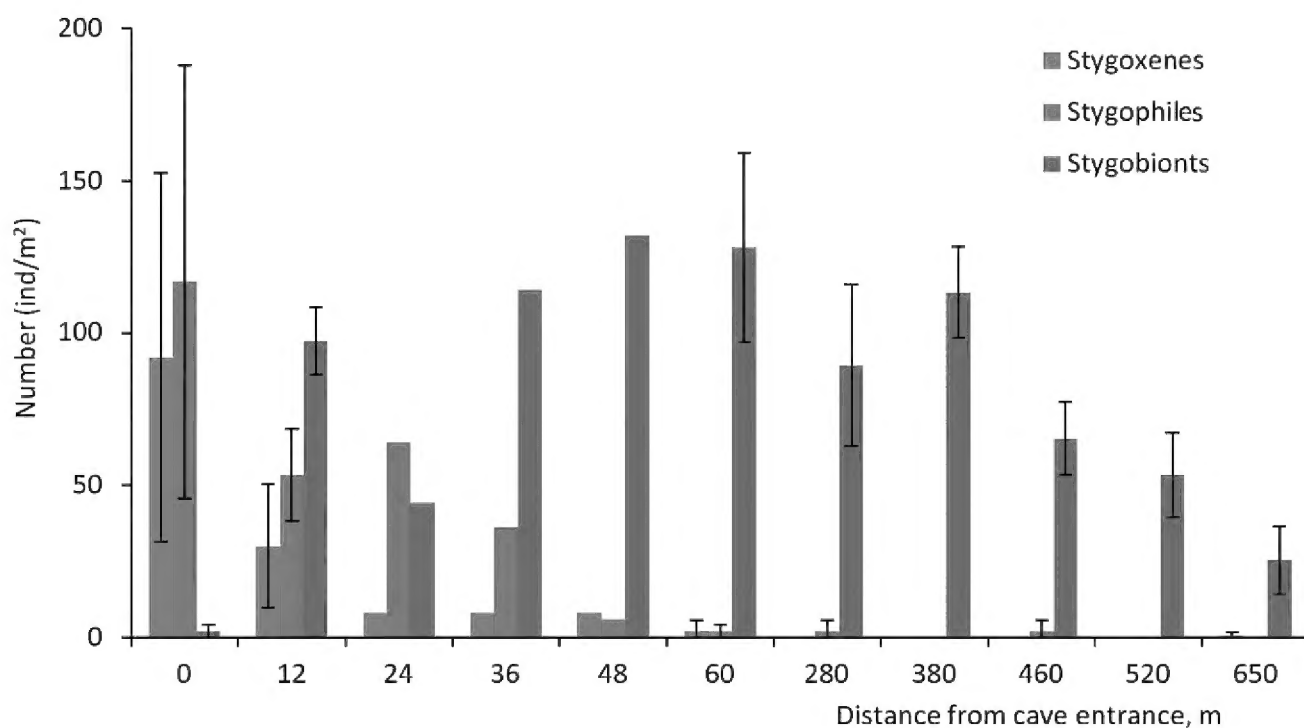


Figure 3. Changes in the number of stygoxenes, stygophiles and stygobionts according to the distance from the cave entrance (2018–2019).



Figure 4. The most numerous stygobionts in the Lower Shakuranskaya Cave **A** *Xiphocaridinella osterloffii* (Juzbaš'jan, 1941) **B** *Pontohoratia birsteini* (Starobogotov, 1962).

Among the stygophiles, the most abundant were a flatworm (*Dugesia taurocaucasica* (Livanov, 1951)) (up to 54 ind/m²), snail (*Tschernomorica caucasica* (Starobogotov, 1962)) (up to 52 ind/m²) and amphipod (*Gammarus* cf. *komareki* (Schaferna, 1922)) (up to 52 ind/m²). These species were associated mainly with the slightly illuminated part of the ecotone zone.

Mayfly larvae *Baetis* cf. *gemellus* Eaton, 1885 (up to 142 ind/m²), and caddisfly larvae *Lithax incanus* (Hagen, 1859) (up to 20 ind./m²) were the most numerous among the stygoxenes. These species were recorded in the ecotone part, and their maximum abundance was observed at the most illuminated station 1.

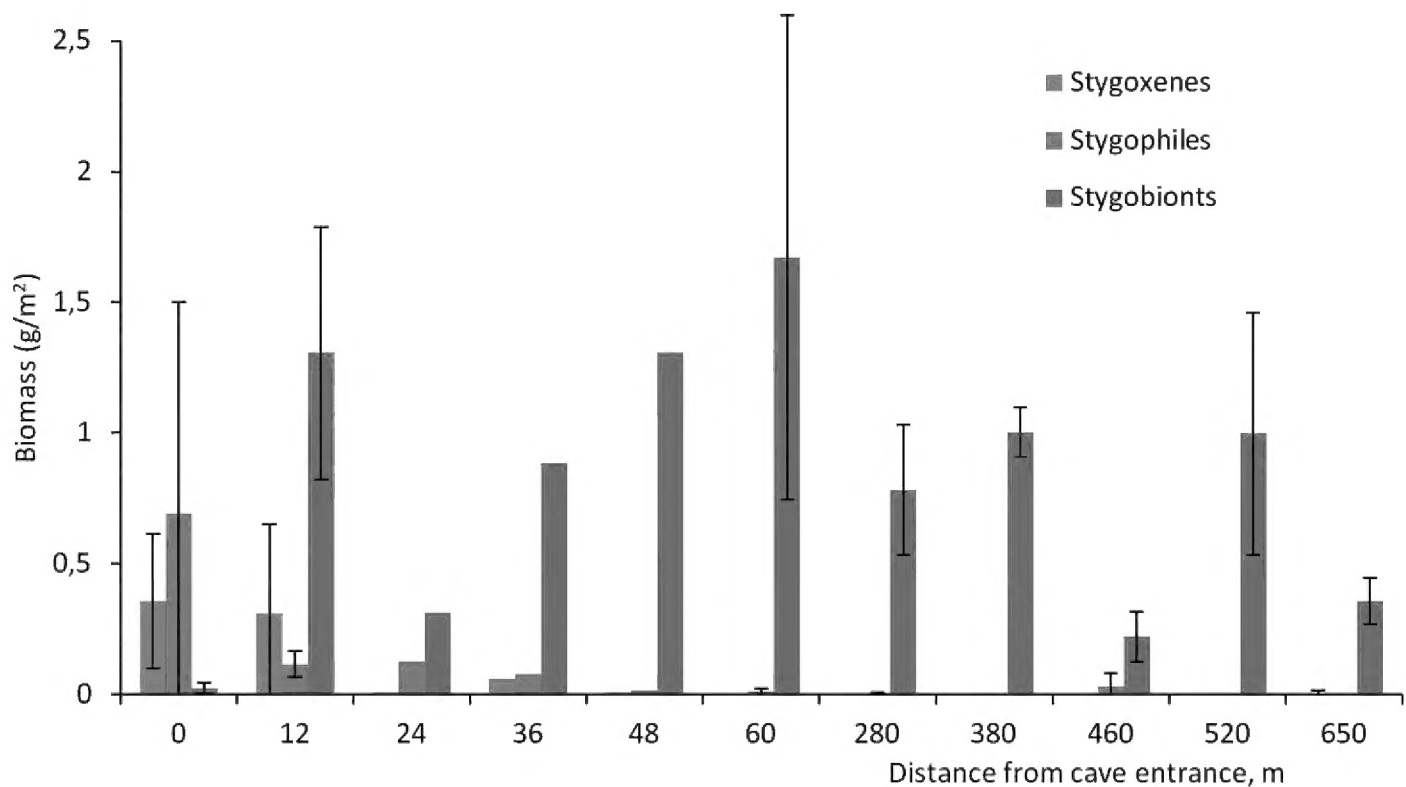


Figure 5. Changes in the biomass of stygoxenes, stygophiles and stygobionts according to the distance from the cave entrance (2018–2019).

The highest biomass values were recorded in the ecotone zone at stations 2 and 3 (Fig. 5). The main contribution to biomass at these stations was from stygobionts. The predominance of stygoxenes and stygophiles over stygobionts in the biomass was noted only outside of station 1. The biomass values recorded for stations located in the ecotone (at a distance less than 100 m from the cave entrance) were higher than those in the more distant parts of the cave (Fig. 3). The lowest biomass values were noted at stations 6 and 8, located at distances of 460 and 650 m from the cave entrance, respectively. It should be noted that *Xiphocaridinella* shrimp accounted for the main part of the biomass at most of the transect stations, including all the stations in the ecotone zone, except for station 1 near the cave entrance (Fig. 6).

Community structure across a gradient of environmental factors

Of the four environmental variables we measured for the whole dataset (year, season, distance, and luminosity), the DistLM analysis identified luminosity and distance as explaining the highest amount (31.7% and 29%, respectively) of the variation in species assemblage structure (Table 3). The set of sequential tests shows whether adding every particular variable contributes significantly to the explained variation. The column labeled “Cumul.” provides a running cumulative total. Thus, these variables explained 55.6% of the variation in the species composition at the observed sampling stations (Fig. 7). Of the variables, only distance and luminosity were statistically significant ($P = 0.001$).

A significant proportion of the species assemblage variations remains unexplained, which is due to the high heterogeneity of the other environmental conditions in the biotopes studied. By taking into account a greater variety of environmental factors, we attempted to conduct a separate, more detailed analysis for the third sampling event

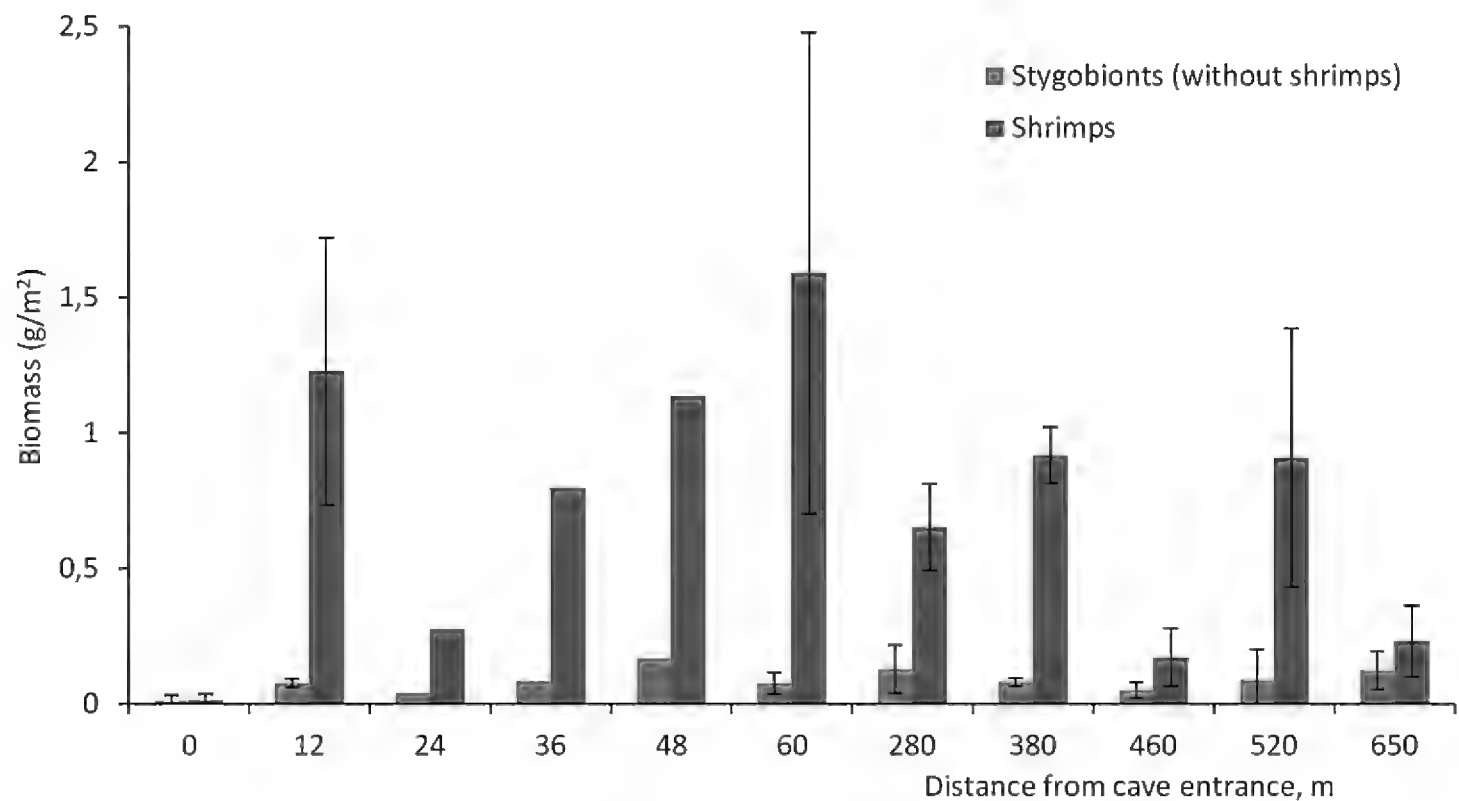


Figure 6. Changes in the biomass of stygobionts according to the distance from the cave entrance (2018–2019).

Table 3. The results from DistLM test, including marginal and sequential tests.

Variable	AICc	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
MARGINAL TESTS							
Distance		16257	10.209	0.001	0.29		
Luminosity		17783	11.612	0.001	0.317		
Year		3256.3	1.5414	0.152	0.051		
Season		2506.7	1.17	0.301	0.045		
SEQUENTIAL TESTS							
+ Season	209.51	2506.7	1.17	0.298	0.045	0.045	25
+ Distance	202.74	15615	9.875	0.001	0.278	0.323	24
+ Luminosity	195.43	11833	10.42	0.001	0.211	0.534	23
+ Year	197.19	1203.5	1.063	0.408	0.0215	0.556	22

Factors with $p < 0.005$ are in bold. AICc – modified Akaike information criterion by which only significant factors in model are selected; SS (trace) – the total sum of squares of the deviations explained with this; Pseudo-F – the multivariate analogue of Fisher's ratio, estimates by how much the sum of squares deviates from; random P – probability of random influence of a factor; Prop. – the proportion of variability which explains each factor (in the marginal tests – without coactions of factors); Cumul. – running cumulative total (percent of the variability explained by the model); res.df – number of degrees of freedom (number of groups allocated by this factor).

in autumn 2019. For this period of research, some additional data were available. The DistLM analysis showed that of all the factors (season, distance from the cave entrance, illumination, maximum depth of the stream, maximum width, flow rate, water temperature °C, total mineralization TDS ppm and pH), only two, the flow rate and pH, were nonsignificant and therefore eliminated (Fig. 8). Among the variables, illuminance, distance and TDS explained the most variation. Overall, the model explained 85.8% of the variation in species composition at the observed sampling stations.

The observed factors affected both the species composition and species richness of organisms in the samples. Using the regression analysis, only the factor of distance was selected as significant (P-value 0.00006). Altogether, 54.4% of the variation in the number of species can be explained by the model. The obtained regression equation

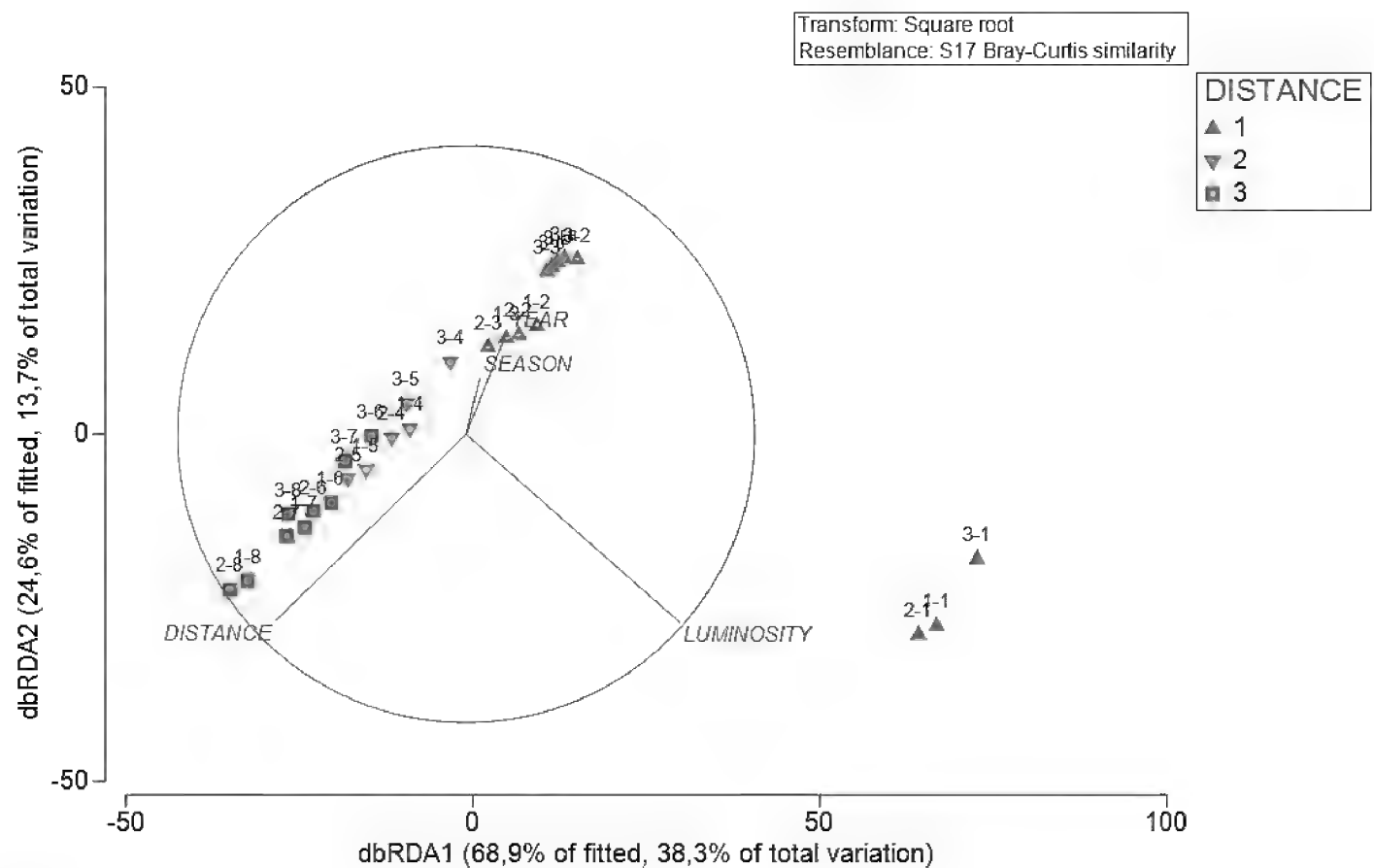


Figure 7. dbRDA ordination for the model of the investigated cave sites (based on Bray–Curtis similarity) factored with distance ranges: 1 – 0–60 m, 2 – 280–380 m, 3 – 460–650 m.

predicts a decrease in the number of species by 0.009184 with a one-meter increase in distance; in other words, a 100-meter decrease in the distance from the cave entrance leads to a one-species drop in the number of species.

Species assemblages

To further illustrate the ordination of the investigated stations according to their species compositions, we used nonmetric MDS, which revealed three groups, i.e., three species assemblages, that were clustered together on the basis of preference for luminosity (Fig. 9). There was a group of stations located at the entrance to the cave, where the illuminance was highest (555 lx, three dots on the left side of the nMDS plot), a group of stations (six dots) in semidarkness and a scatter of dots with a lux value of zero (18 dots on the right side of the nMDS plot).

The CCA plot (Fig. 10) shows the variation in the species assemblages of aquatic invertebrates in accordance with the observed environmental factors. The first ordination axis (axis 1, eigenvalue 0.72081) positively correlated with luminosity and negatively correlated with the distance from the cave entrance. Thus, it reflected the most strongly pronounced gradients of the environmental conditions in the cave, along which stygobiont organisms are gradually replaced with epigeic organisms. The species *G. cf. komareki*, *Agabus guttatus* (Paykull, 1798), *Elmis* sp., *Stenophylax clavatus* (Martynov, 1916), *Tinodes valvatus* Martynov, 1913, *L. incanus*, *B. cf. gemellus* and others, located on the right side of the CCA plot (Fig. 7), are typical of epigeic communities, while

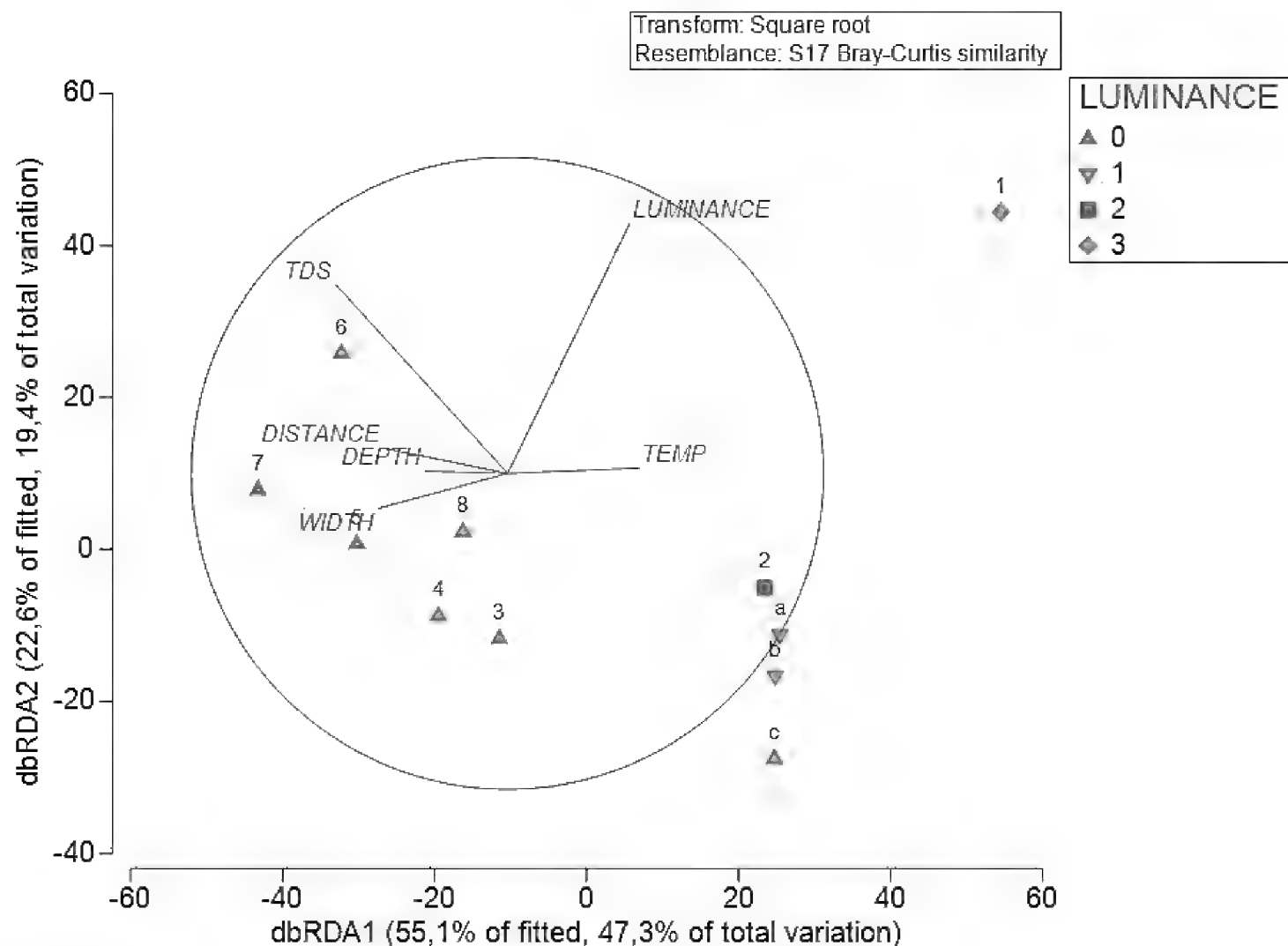


Figure 8. dbRDA ordination for the investigated cave sites during the research in autumn 2019 (based on Bray–Curtis similarity) factored with luminosity ranges: 0 – 0 lx, 1 – 0.07–2.7 lx, 2 – 13.17 lx, 3 – 555 lx.

the species *Stylodrilus* sp., *Esenia* sp., *C. horatieformis*, *C. schakuranica*, *C. sp.*, *Z. yakovi*, *Xiphocaridinella falcirostris* Marin, 2020 and others on the left side are typical stygobionts. The second CCA axis, axis 2 (eigenvalue 0.26147), was positively correlated with the season of research; however, its contribution to explaining the variability in the structure of species assemblages was extremely low. Apparently, the location of species along this axis primarily characterized the rare species found in only one of the temporal surveys.

The main characteristics of the three identified species assemblages of macrozoobenthic organisms are presented below:

1. Assemblage of epigeal species near the cave entrance area. This community was characterized by the predominance of epigeal organisms and abundant stygophilic taxa. Larvae of amphibiotic insects (*B. cf. gemellus* and *L. incanus*) and epigeal Amphipoda (*G. cf. komareki*) were dominant (66% of the total fauna). Stygophilic snails (*T. caucasica*) and flatworms (*D. taurocaucasica*) were also abundant (27%). Stygobionts (two species) were very rare, accounting for only 1% of the total number of macrozoobenthic species, and they must have been driven from the remote parts of the caves. The number of species totaled 15.

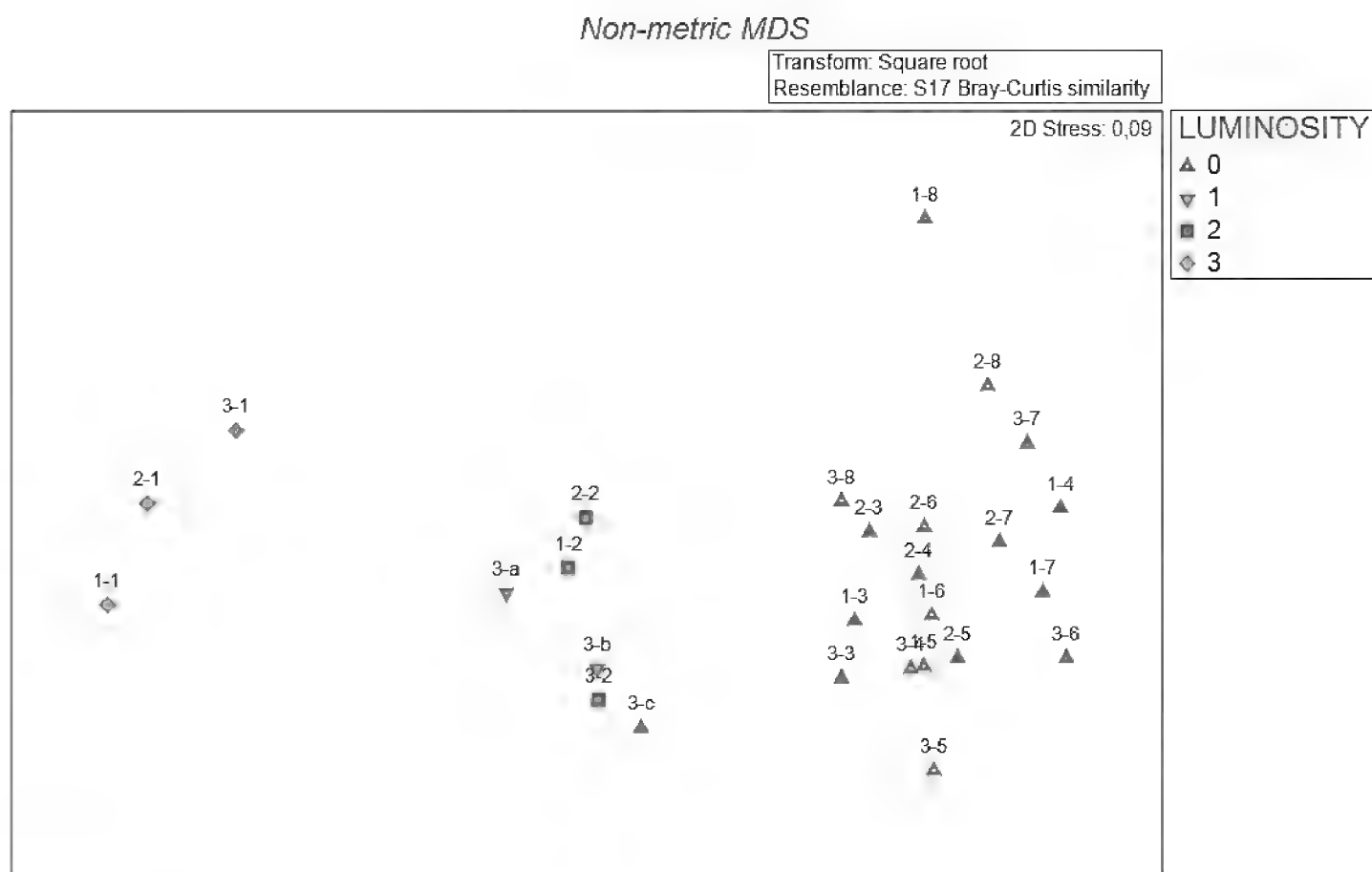


Figure 9. The two-dimensional nMDS ordination of the investigated cave sites, based on Bray–Curtis similarities (stress = 0.09) and factored with luminosity: 0 – 0 lx, 1 – 0.07–2.7 lx, 2 – 13.17 lx, 3 – 555 lx. Dots are labeled: first number – season/year of research: 1 – winter 2018, 2 – spring 2018, 3 – autumn 2019; second number – no of sampling station.

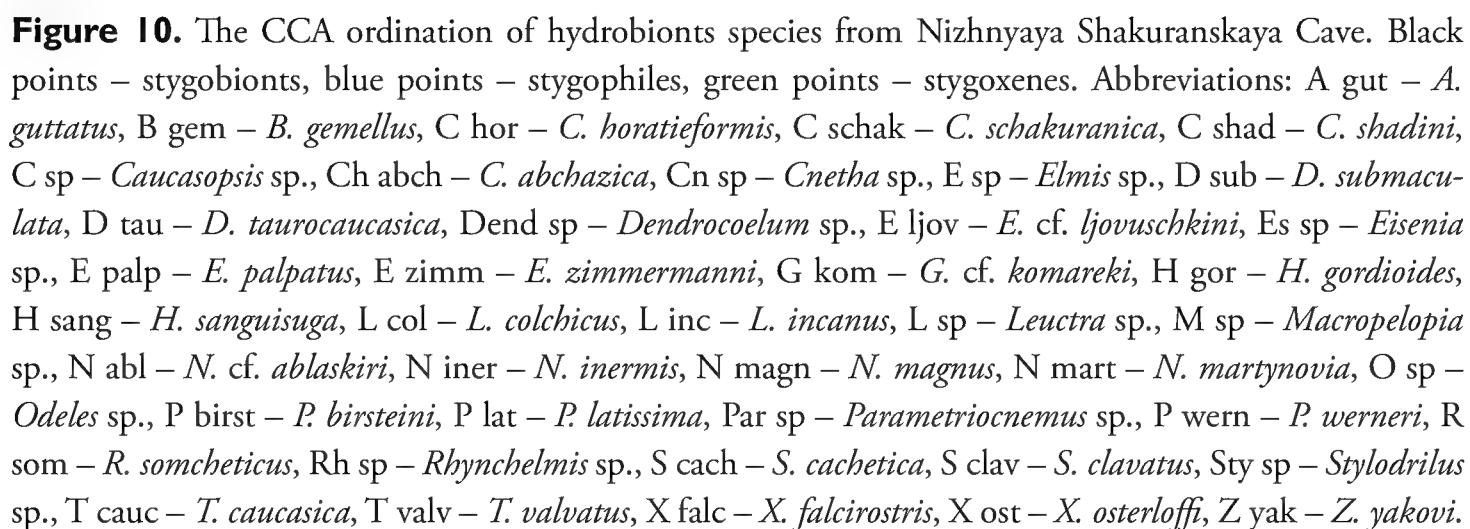
2. Assemblage of stygobiont species in remote (> 40 m from the entrance) parts of the cave outside the photic zone. Stygobiont oligochaetes (*Stylodrilus* sp.), amphipods (*Z. yakovi*), shrimp (*Xiphocaridinella* spp.) and snails (*P. birsteini* and *C. schakuranica*) formed the bulk of the community (81% of the total abundance). Stygoxenic species were rare (1% < of total number). The number of species totaled 25.

3. Mixed assemblage of the cave ecotone (first 40 m from the entrance to the border of the photic zone). This assemblage is transitional between the two previously described assemblages. The common species include both stygoxenes (*B. cf. gemellus*, 8% of the total fauna) and stygophiles (*T. caucasica* and *D. taurocaucasica*, 29%) as well as stygobionts (*P. birsteini* and *Xiphocaridinella osterloffii* (Juzbaš'jan, 1941), 41%). The total number of species was the highest here (27 species).

Discussion

Main characteristics of stygobiont fauna

A total of 14 stygobiont species were found in the Lower Shakuranskaya Cave in 2018–2019, and this number is comparable to the variety of stygophiles (10) and stygoxenes (18). Earlier (in 2012), 14 species of stygobionts were observed in this cave (Chertoprud et al. 2016). Most were found in the present study. Thus, the general list



The two major groups in the stygobiont assemblage were gastropods belonging to *Caucasopsis*, *Caucasogeyeria*, and *Pontohoratia* (f. Hydrobiidae) and shrimp belonging to *Xiphocaridinella* (f. Atyidae) (Fig. 4). Mollusks were the most abundant group in terms of the number of individuals, while shrimp comprised the main biomass (Fig. 6).

Significant changes in the dominance structure and qualitative and quantitative characteristics along the Lower Shakuranskaya Cave gallery occur. Thus, three types of

macrozoobenthic assemblages, continually changing each other, were indicated. The ecotone consists of mixing assemblages in which stygoxenes, stygophiles and stygobionts are abundant simultaneously. The abundance and species richness of stygobionts increase from the onset of the ecotone zone, peak at 50–60 m from the cave entrance and decrease further into the cave (Table 2, Figs 3, 5).

The peak abundance in the ecotone may be related to bottom sedimentation and food availability. The bottom in the ecotone zone is covered with rocky soils with a large number of microcavities forming favorable habitats for organisms. In contrast, substrate in deeper parts comprises calcified hump dams and baths without suitable shelters. Some other researches demonstrated positive relationships between environmental heterogeneity and the diversity of aquatic organisms in cave and surface streams (Palmer et al. 2010; Pellegrini et al. 2018). Furthermore, the amount of organic matter monotonically decreases from the ecotone zone towards deeper parts of the cave. The Lower Shakuranskaya Cave is oligotrophic (Amelichev et al. 2007), whereas the ecotone zone seems to be less food deprived because of the inflow of plant detritus and filamentous algae in the presence of light. The food that may be safely accessed through the microcavities might attract stygobionts to the boundary of the aphotic zone.

Apart from the beneficial aspects of the ecotone zone, stygobionts can passively drift out from the cave with water currents. Indeed, stygobionts are occasionally found outside the caves as a result of seasonal floods. For example, *Xiphocaridinella* shrimp (Marin and Sokolova 2014) and the snail *Radomaniola curta germari* (Frauenfeld, 1863) (Perić et al. 2018) are found in epigean streams during spring and autumn high water. Although intuitively logical, these explanations need to be considered with care. It has been observed that a number of stygobionts (for example, stygobiont amphipods) that live at the border of belowground and aboveground environments can actively avoid the water current and illuminated areas, thus resisting being transported from cave biotopes (Borowsky 2011; Fišer et al. 2016).

It must be noted that the abundance and biomass of stygobionts in our study were not extremely low in the deeper and oligotrophic parts of the studied cave (more than 200 m), where species apparently feed on the microbial community containing heterotrophic and, to a lesser extent, chemoautotrophic bacteria (Kováč 2018). This food source might also explain the dominance of the cave shrimp and gastropods. The Atyidae family includes pereopods adapted to collecting bacterial biofilms due to their specific bristle armament (Page et al. 2007). Most likely, numerous gastropod mollusks can be considered consumers of biofilms, which they scrape off underwater fouled surfaces with their radula. Perhaps bacterial communities serve as one of the main food sources for stygobiont fauna in the Lower Shakuranskaya Cave. While these hypotheses need to be tested with stable isotope analysis, we acknowledge that the ecotone zone might act as a food attractant mainly to less frequent species and to a lesser extent to collectors of biofilms.

Thus, this study confirms the hypothesis about the increase in species richness and abundance of aquatic organisms in the ecotone zone (Prous et al. 2004, 2015; Culver 2005). Our hypothesis that the ecotone macroinvertebrate assemblages may significantly differ from the assemblages of the remote cave parts was confirmed.

Active and passive ways to penetrate epigeic species in cave communities

The entrance of the Lower Shakuranskaya Cave is large (approximately 70 m²). Adult amphibiotic insects were not found inside the cave, and their larvae usually do not occur further than 60 m deep. Only certain stygoxenes (*Haemopsis sanguisuga* (Linnaeus, 1758), *Ernodes palpatus* (Martynov, 1909), *Schizopelex cachetica* Martynov, 1913 and *Parametriocnemus* sp.) can penetrate through the photic zone. The active penetration of stygophiles and stygoxenes further than the ecotone zone indicates their ability to actively migrate against the flow. Most likely, the intensity of these migrations is determined by the presence of an available food, as in the case of the leech *H. sanguisuga* (Linnaeus, 1758), which feeds on stygobiont oligochaetes.

The finding of stygoxenic and stygophilic insect larvae at a great distance from the cave entrance may be a consequence of drift (i.e., the movement of benthic organisms with the current). This phenomenon is widespread in watercourses and plays a significant role in the distribution of benthos in mountain regions (Brittain and Eikeland 1988; Naman et al. 2016). In the investigated cave, larvae of the Ephemeroptera *Electrogena zimmermanni* (Sowa, 1984) and Plecoptera *Leuctra* sp. were found at distances greater than 400 m from the entrance. These species have previously been noted in epigeic watercourses of the western Caucasus (Chertoprud et al. 2016, 2020). The most likely method of larval penetration in the cavities is passive drift with water through the rock cracks and karst tunnels. The greatest intensity of drift was observed during flood events (Perić et al. 2018).

In the context of global climate changes affecting organic matter flows in ecosystems, a significant transformation of cave ecosystems can be expected (Humphreys 2018). It has been suggested that ongoing warming of the climate may cause an increase in the nutrient status of cave watercourses, which can lead to more intensive penetration of epigeic species into underground cavities. It was observed previously that stygophiles and stygoxenes actively settle underground habitats in caves with organic pollution (Sousa-Silva et al. 2012; Venarsky et al. 2012, 2018). Establishment of long-term observations of aquatic fauna in model caves will enable the assessment of the value of biospeleology for monitoring global climatic processes.

Conclusion

In the Lower Shakuranskaya Cave, 42 species of aquatic invertebrates occurred: 14 – stygobionts, 10 – stygophiles, and 18 – stygoxenes. The species richness and abundance of stygobionts were the greatest near the boundary of the photic zone and gradually decreased both further into the cave cavity and up to the exit from it. In the cave, the distributions of most stygoxenes and stygophilic species were limited to the illuminated ecotone zone. The main factors regulating the spatial distributions of macrozoobenthic organisms were the distance from the cave entrance and the light intensity (illuminance). The greatest species richness and abundance of fauna were noted at sta-

tions in the shaded ecotone, where stygobionts, stygophiles and stygoxenes co-occur. The most likely reasons for this scenario are the higher abundance of food resources for aquatic invertebrates, the removal of stygobionts by the water current, and the possibility of faunal epigean elements penetrating the ecotone zone.

Acknowledgements

The study is supported by the Russian Foundation for Basic Research (RFBR) (grant 20-04-00803_A). This research was performed according to the Development program of the Interdisciplinary Scientific and Educational School of M.V. Lomonosov Moscow State University "The future of the planet and global environmental change".

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